

## 2008 Annual Report

PI: John Frost

Performer: Michigan State University

Award#: N000140210725

### a. Scientific and Technical Objectives

Three challenges confront microbial synthesis of phloroglucinol using heterologous expression of *Pseudomonas fluorescens phlD* in *Escherichia coli*: (a) the specific activity and catalytic lifetime of *PhlD*; (b) the availability of malonylCoA (the substrate for *phlD*-encoded phloroglucinol synthase); and (c) toxicity of phloroglucinol towards the *E. coli* host used to express plasmid-localized *phlD*. Challenges (a) and (b) were the focus of MURI activity.

### b. Approach

Three different promoters were examined for expression of *PhlD* in *E. coli*. Three different strains of *E. coli* were examined as hosts for heterologous expression of *phlD*. The role of malonylCoA substrate as a limiting factor in phloroglucinol synthesis was examined using heterologous expression in *E. coli* of a malonylCoA synthetase and a malonic acid transporter protein along with supplementation of cultures with malonic acid.

### c. Concise Accomplishments

Concentrations of phloroglucinol synthesized under fermentor-controlled conditions in the absence of resin-based extraction were separately determined when *phlD* was expressed from a *Tac*, *T5* and *T7* promoter. The highest concentrations of phloroglucinol were synthesized when *phlD* was expressed from a *T7* promoter. *E. coli* K12, *E. coli* B and *E. coli* C41 were examined as hosts for expression of plasmid-localized *phlD*. *E. coli* C41 was the best host for the fermentor-controlled synthesis of phloroglucinol in the absence of resin-based extraction. Phloroglucinol synthesis increased when the culture medium of an *E. coli* construct expressing *matBC* was supplemented with malonic acid.

### d. Expanded Accomplishments

Phloroglucinol is synthesized by heterologous expression in *E. coli* of the *phlD* gene isolated from *P. fluorescens* Pf-5. The *phlD* gene is plasmid-localized and expression controlled by an IPTG-inducible *T7* promoter. Plasmid maintenance is achieved using nutritional pressure. The host *E. coli* strain's genomic *serA* locus, which encodes an enzyme involved in serine biosynthesis, is inactivated. To grow in minimal salts medium in the absence of serine supplementation, the host *E. coli serA* mutant must stably maintain the *phlD*-encoding plasmid that also carries a *serA* insert.

*E. coli* BL21*serA*/pMX1.213(p*Tac-phlD*) and *E. coli* BL21*serA*/pMX1.227(p*T5-phlD*) were constructed and synthesized phloroglucinol concentrations compared to *E. coli* BL21*serA*/pJA3.131A(p*T7-phlD*) under fermentor-controlled conditions in the absence of resin-based extraction. Phloroglucinol was synthesized in concentrations of 2.3 g/L, 2.3 g/L, and 4.1

Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE <b>2008</b>		2. REPORT TYPE <b>N/A</b>		3. DATES COVERED <b>-</b>	
4. TITLE AND SUBTITLE <b>2008 Annual Report - Award#: N000140210725</b>				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) <b>Michigan State University</b>				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT <b>Approved for public release, distribution unlimited</b>					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT <b>SAR</b>	18. NUMBER OF PAGES <b>3</b>	19a. NAME OF RESPONSIBLE PERSON
a. REPORT <b>unclassified</b>	b. ABSTRACT <b>unclassified</b>	c. THIS PAGE <b>unclassified</b>			

g/L by BL21*serA*/pMX1.213(pTac-*phlD*), BL21*serA*/pMX1.227(pT5-*phlD*), and BL21*serA*/pJA3.131A(pT7-*phlD*), respectively.

*E. coli* W3110(DE3)*serA*, *E. coli* BL21(DE3)*serA*, and *E. coli* C41(DE3)*serA* were evaluated as hosts for expression of pJA3.131A(pT7-*phlD*). W3110 is an *E. coli* K12 strain while BL21 is an *E. coli* B strain. In ethanol fermentations, *E. coli* B strains typically are preferred hosts due to their greater resistance to ethanol and cellulosic hydrolysates. C41 is another *E. coli* B strain developed by Lucigen to withstand expression of toxic proteins. These phloroglucinol-synthesizing constructs were cultured under fermentor-controlled conditions at 2 L scale in the absence of resin-based extraction. Phloroglucinol was synthesized at concentrations of 3.4 g/L, 4.1 g/L, and 4.0 g/L by W3110(DE3)*serA*/pJA3.131A, BL21(DE3)*serA*/pJA3.131A, and C41(DE3)*serA*/pJA3.131A, respectively. Both W3110(DE3)*serA*/pJA3.131A and BL21(DE3)*serA*/pJA3.131A produce a substantial amount of foam during fermentor-controlled cultivation. By contrast, this foaming problem is absent during fermentor-controlled cultivation of C41(DE3)*serA*/pJA3.131A. Based on concentration of phloroglucinol synthesized and ease of cultivation under fermentor-controlled conditions, *E. coli* C41(DE3)*serA* has become the preferred strain for examining phloroglucinol production.

Plasmid pJA3.131-matBC was constructed as part of the effort to determine if malonylCoA availability limits microbial synthesis of phloroglucinol. *Rhizobium leguminosarium* bv *trifolii* was the source of *matB*, which encodes malonylCoA synthetase and *matC*, which encodes a protein capable of transporting malonic acid into the cytoplasm from the culture medium. *E. coli* W3110(DE3)*serA*/pJA3.131-matBC was constructed to evaluate the impact of malonic acid supplementation on the concentrations of phloroglucinol synthesized from glucose. MatC was preceded to transport the malonic acid added to the culture medium into the *E. coli* cytoplasm where MatB catalyzes the conversion of the transported malonic acid into malonylCoA. MalonylCoA is then the substrate for phloroglucinol synthase.

*E. coli* W3110(DE3)*serA*/pJA3.131-matBC was cultured under fermentor-controlled conditions in the absence of resin-based extraction both with and without malonic acid supplemented at a concentration of 4 g/L. In the absence of malonic acid supplementation, *E. coli* W3110(DE3)*serA*/pJA3.131-matBC synthesized 0.2 g/L of phloroglucinol. In the presence of malonic acid supplementation, *E. coli* W3110(DE3)*serA*/pJA3.131-matBC synthesized 0.6 g/L of phloroglucinol. The amount of phloroglucinol synthesized by *E. coli* W3110(DE3)*serA*/pJA3.131-matBC is much less than the 3.4 g/L synthesized by *E. coli* W3110(DE3)*serA*/pJA3.131. This likely reflects the toxic impact on *E. coli* attendant with unregulated transport into its cytoplasm of malonic acid, which is a central metabolite essential to cell wall biosynthesis. Nonetheless, the higher concentration of phloroglucinol synthesized by *E. coli* W3110(DE3)*serA*/pJA3.131-matBC with malonic acid supplementation suggests that in vivo availability of malonic acid may be a limiting factor during microbial synthesis of phloroglucinol.

e. Work Plan

f. Major Problems/Issues

g. Technology Transfer

#### h. Foreign Collaborations and Supported Foreign Nationals